METHOD AND APPARATUS FOR SAMPLE INJECTION IN MICROFABRICATED DEVICES

This patent application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Patent Application Serial No. 60/267,474, filed on February 9, 2001.

BACKGROUND OF THE INVENTION

1. Cross-Reference

U.S. Patent Application No._____ entitled METHOD AND APPARATUS FOR REPRODUCIBLE SAMPLE INJECTION ON MICROFABRICATED DEVICES, concurrently filed on February 11, 2002 (Attorney Docket No.: 1093/202), which is assigned to MicroChem Solutions, Inc., the assignee of the present invention, and which is fully incorporated by reference herein.

2. Field of the invention

The present invention relates generally to miniature instrumentation for conducting chemical reaction and/or bio-separation, and diagnostics and/or analysis related thereto, and more particularly, to the introduction of samples to the chemical reaction and/or bio-separation channels in microfabricated devices.

3. Description of Related Art

Bioanalysis, such as DNA analysis, is rapidly making the transition from a purely scientific quest for accuracy to a routine procedure with increased, proven dependability. Medical researchers, pharmacologists, and forensic investigators all use DNA analysis in the pursuit of their tasks. Yet due to the complexity of the equipment that detects and measures DNA samples and the difficulty in preparing the samples, the existing DNA analysis procedures are often time-consuming and expensive. It is therefore desirable to reduce the size, number of parts, and cost of equipment, to make easy sample handling during the process.

One type of DNA analysis instruments separates DNA molecules by relying on electrophoresis. Electrophoresis techniques could be used to separate fragments of DNA for genotyping applications, including human identity testing, expression analysis, pathogen detection, mutation detection, and pharmacogenetics studies. The term electrophoresis refers to the movement of a charged molecule under the influence of an electric field. Electrophoresis can be used to separate molecules of different electrophoretic mobilities in a given separation medium. DNA fragments are one example of such molecules.

There are a variety of commercially available instruments applying electrophoresis to analyze DNA samples. One such type is a multi-lane slab gel electrophoresis instrument, which as the name suggests, uses a slab of gel on which DNA samples are placed. Electric charges are applied across the gel slab, which cause the DNA sample to be separated into DNA fragments of different masses.

Another type of electrophoresis instruments is the capillary electrophoresis instrument. Capillary electrophoresis can be considered as one of the latest and most rapidly expanding techniques in analytical chemistry. Capillary electrophoresis refers to a family of related analytical techniques that uses very strong electric fields to separate molecules within narrow-bore capillaries (typically 20-100 um internal diameter). Capillary electrophoresis techniques are employed in seemingly limitless applications in both industry and academia.

A variety of molecules can be separated by capillary electrophoresis techniques. Sample types include simple organic molecules (charged or neutral), inorganic anions and cations, peptides, oligonucleotides, and DNA sequence fragments. Since the introduction of commercial instrumentation in 1988, the inherent capabilities of capillary electrophoresis and its various modes of operation have been widely demonstrated. Major advantages of capillary electrophoresis include high separation efficiency, small sample and reagent consumption, and low waste generation. The sample fragments in capillary electrophoresis are often analyzed by detecting light emission (e.g., from radiation induced fluorescence) or light absorption associated with the sample. The intensities of the emission are representative of the concentration, amount and/or size of the components of the sample.

Specifically, in capillary electrophoresis, separation is performed in small capillary tubes to reduce band broadening effects due to thermal convection and hence improve resolving power. By applying electrophoresis in a capillary column carrying a buffer solution, the sample

size requirement is significantly smaller and the speed of separation and resolution can be increased multiple times compared to the slab gel-electrophoresis method. Only minute volumes of sample materials, typically less than 20 nanoliters, are required to be introduced into the separation capillary column.

It was mentioned in The Journal of Chromatography, 452, (1988) 615-622, that sample valves are the most suitable sampling method for capillary electrophoresis. The limitation of this method is the large sampling volume. A rotary injection valve has been used in capillary electrophoresis with a sampling volume of 350 nanoliters. The results have been reported in Anal. Chem. 59, (1987) 799. This volume is too large to be used for high-resolution separations. Later, an internal loop injection valve with an injection loop volume of $\geq$ 20 nL has become commercially available, but connecting capillaries to this valve is too much of a challenge and consequently it is not often used in capillary electrophoresis.

Current practical techniques for sample injection in capillary electrophoresis include electromigration and siphoning of sample from a container into one end of a separation column. For the siphoning injection technique, the sample reservoir is coupled to the inlet end of the capillary column and is raised above the buffer reservoir that is at the exit end of the capillary column for a fixed length of time. The electromigration injection technique is effected by applying an appropriate polarized electrical potential across the capillary column for a given duration while the entrance end of the capillary is in the sample reservoir. For both sample injection techniques the input end of the analysis capillary tube must be transferred from a sample reservoir to a buffer reservoir to perform separation. Thus, a mechanical manipulation is involved. It is also difficult to maintain consistency in injecting a fixed volume of sample by either of these techniques, as the sample volume injected are susceptible to changes in sample viscosity, temperature, etc., thereby resulting in relatively poor reproducibility in injected sample volumes between separation runs. Electromigration additionally suffers from electrophoretic mobility-based bias.

Electrophoresis based on microfabricated chips possesses many unique advantages over conventional capillary electrophoresis. One of them is the so-called "differential concentration" effect for separation of DNA sequencing fragments. For sequencing using conventional capillary gel electrophoresis, the signal intensity of separated fragment has an exponential profile against fragment size. That is, very high signal intensities for short fragments and very low for

large fragments. Often, the readlength of DNA sequencing is limited by the low signal intensity rather than the resolution for the long fragments. This exponential profile also requires a wide dynamic range for detection.

Capillary electrophoresis on microchips is an emerging new technology that promises to lead the next revolution in chemical analysis. It has the potential to simultaneously assay hundreds of samples in minutes or less time. Microfluidic chips used in electrophoretic separations usually have dimensions from millimeters to decimeters. The largest electrophoretic separation chip so far has a substrate having dimensions of 50-cm x 25-cm, which was disclosed in Micro Total Analysis Systems 2001, 16-18. These microfluidic platforms require only nanoliter or picoliters volumes of sample, in contrast to the microliter volumes required by other separation technologies. These samples may potentially be prepared on-chip for a complete integration of sample preparation and analysis functions. The rapid analysis combined with massively parallel analysis arrays could yield ultrahigh throughputs. These features make microchips an attractive technology for the next generation of capillary electrophoresis instrumentation.

These microchips are prepared using microfabrication techniques developed in the semiconductor industry. Capillary channels are fabricated in microchips using, for example, photolithography or micromolding techniques. Microchips have been demonstrated for separations of amino acids, DNA restriction fragments, PCR products, short oligonucleotides, and sequencing ladders.

For capillary electrophoresis separation on microchips, samples are usually introduced using either cross-channel or double-T sample injectors. The cross-channel injector has been disclosed in U.S. Patent No. 6,001,229. As illustrated in Figure 1a, the cross-channel injector is formed by orthogonally intersecting the separation-channel **6** with a cross-channel **5** and **5a** connecting the sample reservoir **1** to an analyte waste reservoir **2**. To load sample to the separation-channel **6**, analytes are electrophoresed (e.g., by electrokinetic forces) from the sample reservoir **1** to the analyte waste reservoir **2**, filling the whole cross-channel **5** including the intersection region **7**. When an electric potential is applied to cathode reservoir **3** and anode reservoir **4** along the separation-channel **6** after analytes have been loaded into the intersection region **7**, the analytes residing in the intersection region **7** are electrokinetically driven down the separation-channel **6** to perform electrophoretic separation.

In the sample loading process, as analytes migrate across the intersection region **7** analytes disperse orthogonally into the separation-channel **6** due to the electric field distortion and molecular diffusion. This degrades the resolving power and makes the separation irreproducible. To overcome this dispersion, selected voltages are applied to the cathode and anode reservoirs **3** and **4** such that buffer electrolytes, along with the dispersed analytes, are electrokinetically driven to the intersection region **7** and ultimately to the analyte waste reservoir **2**. Therefore analyte dispersion is suppressed. This is called a "pinched" injection mode.

A double-T injector on microchips has been disclosed in U.S. Patent No. 6,280,589. In a double-T injector (referring to Figure 1b), the sample channel across the separation channel **6** is divided by the separation-channel into two segments **8** and **9** that are offset by a given distance along the separation channel **6**. If the channel connecting the sample reservoir **1** and analyte waste reservoir **2** is still considered the "cross-channel", the offset segment **10** is shared by the "cross-channel" and the separation-channel **6**. Similar to the cross-channel injector in Figure 1a, sample is loaded by electrophoresis, from the sample reservoir **1** to the analyte waste reservoir **2**, filling the cross-channel segments **8** and **9** and the offset segment **10**. As an electric potential is applied to cathode and anode reservoirs **3** and **4** across the separation-channel **6** (including the offset segment **10)** after analytes have been loaded in the off-set segment **10**, the analytes residing in the offset segment region **10** are electrokinetically driven down the separation-channel **6** to perform electrophoretic separation. Double-T injectors also suffer from dispersion of analytes into the separation channel. "Pinched" injection mode is usually used to suppress this problem, as discussed in Anal. Chem. 71 (1999) 566-573.

Precise control of the potentials on multiple electrodes in reservoirs **1**, **2**, **3** and **4** is critical to achieving desired and reproducible results for either cross-channel or double-T injectors when a "pinched" injection mode is employed. These potentials are balanced and calibrated normally using a standard sample until reproducible results have been obtained. However, when samples of different ionic strength and viscosity are to be analyzed, that calibrated potential balance for the device is no longer applicable, and consequently giving rise to undesired and/or irreproducible results.

For sequencing separation on chips with a cross or a double-T sample injector, a uniform signal intensity profile is typically obtained. The mechanism has been illustrated in the Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 5369-5374. During injection, sample is electrophoresed

through the cross channel to the offset segment **10** (referring to Figure 1b). This electrophoresis of DNA fragments provides differential enrichment of sequencing fragments. Little change in concentration will occur at the sample/gel interface for small DNA fragments and inorganic ions because their electrophoretic mobilities are similar in free solution and in sieving matrix. On the other hand, a considerable increase in the steady-state concentration will occur at the sample/gel interface for the large fragments because of their reduced mobility in the gel. These results in a concentration compensation for large fragments. Concentrations of large fragments are always lower than those of small fragments in a typical sequencing sample. A uniform intensity profile is therefore generated.

Another advantage of microchips is to use cross-channel **5** or **8** (referring to Figure 1) to perform sample preseparation or cleanup. Taking DNA sequencing for example, when sample is electrophoresed through the cross channel **5** to the intersection region **7** or segment **8** to the offset segment **10**, at an optimized injection time, the majority of the fragments have reached a steady-state concentration in the intersection region **7** or segment **10**, while large template and enzyme molecules are still migrating in the cross channel **5** or **8**. When voltages are switched to separation, only the fragments in the injector are injected into the separation channel during the separation, while DNA template and enzyme contaminants were removed from the separation channel. Removal of these large molecules has been reported essential to achieve high quality separations. In capillary gel electrophoresis (CGE), they are removed using offline membrane filters.

T-injectors may be used for sample introduction on microchips as well. In this scheme (referring to Figure 1c), the analyte waste reservoir **2** and the channel **9** between the separation-channel **6** and analyte waste reservoir **2** in Figure 1b are eliminated. Analytes are electrophoresed from the sample reservoir **1** through the half "cross-channel" **8** directly into the separation-channel **6**. Since the other half of the "cross-channel" **9** is omitted, all analytes exit the half "cross-channel" **8** enter and build up in the separation-channel **6** as the sample loading process continues.

However, there are two major problems associated with the T-injector. The first problem is the augmented electrophoretic mobility-based bias. In a normal electrokinetic injection process of capillary electrophoresis, as the sample inlet end of a separation capillary is dipped directly into the sample solution and all analytes migrate into the separation capillary

simultaneously, the electrophoretic mobility-based bias equals to the ratio of their electrophoretic mobilities. In this T-injection scheme, the sample reservoir **1** and the inlet end of the separation-channel **6** are separated by the half "cross-channel" **8**. Fast-moving analytes have already migrated into the separation-channel **6** when slow-moving analytes are still migrating in the half "cross-channel" **8**. As a result, fast-moving analytes are more preferentially introduced in T-injectors than in conventional capillary electrophoresis and therefore the electrophoretic mobility-based bias is augmented.

The second problem is the difficulty in precisely controlling a finite amount of analytes into the separation-channel **6**. This problem is associated with the variation of length of the half "cross-channel" **8**. In a microchip fabrication process, channels are photolithographically created and can be very precisely arranged. The reservoirs are holes drilled or physically attached and their positions and dimensions cannot be reproducibly and precisely produced. In T-injection schemes, the quantity of the analytes injected into the separation-channel **6** is normally controlled through timing of the applied electrical potential. Because analytes going to the separation-channel **6** have to pass through the half "cross-channel" **8**, it is a significant challenge to attempt to control the timing so that only a given finite amount of analytes is allowed to migrate into the separation-channel **6**. Variation of the length of this channel makes the problem even more challenging.

It is therefore desirable to develop a robust and automated sample injection scheme for a microfabricated device, which would overcome the limitations in the prior art. In addition, prior art systems had not adequately addressed the issues and challenges relating to interfacing microfluidic channels with real-world sample and reagent solutions before the full benefits of microfluidic systems can be realized. It is highly desired a sample injection schemes that will facilitate convenient and automated interfacing.

SUMMARY OF THE INVENTION

The present invention provides a simplified off-column sample injection scheme for introducing samples into micro-channels for separation, chemical reaction, etc. (generally referred to as reaction channels or columns) in microfabricated devices, which overcomes the drawback of the prior art. The sample introduction schemes can be used for variety of applications, including integrated microfluidic systems for chemical analysis and sensing, and analytical separation techniques such as capillary electrophoresis, capillary electrochromatography, microcolumn liquid chromatography, flow injection analysis, and field-flow fractionation.

In one aspect of the present invention, off-column sample injection is effected by introducing sample from a sample reservoir provided on the substrate of the microfabricated device into a reaction channel via a constricted channel or opening interface to prevent sample diffusion, improve structural dimension control in fabrication, and reduce augmented electrophoretic mobility-based bias, as compared to the prior art T-injectors. In one embodiment, a relatively short and narrow connection-channel is used to interface between the sample channel and the reaction channel. In another embodiment, a pinhole is used to interface between the sample channel and the reaction channel. In yet another embodiment, a combination of a narrow connection-channel and pinhole. In a further embodiment of the present invention, off-column sample injection is effected by introducing sample from a sample reservoir that is provided outside the substrate of the microfabricated device. A through-hole is provided in the substrate to facilitate introduction of sample into the reaction channel. When the through hole is used as a sample reservoir, it also facilitates convenient cleaning of the sample reservoir.

In another aspect of the present invention, a microfabrication process is developed to provide a pinhole to connect a sample-channel to a reaction channel.

In a further aspect of the present invention, the free-end of a capillary tube connected to the sample-channel is moved alternatively to a sample and an auxiliary solution to bring multiple samples in series to the vicinity of a reaction channel for convenient sample introduction and high-throughput assays.

The operations of the various embodiments of the present invention are controlled by a controller to accomplish the functions recited herein.

In accordance with the sample injection schemes of the present invention, it is not required to balance multiple potentials during the process of sample introduction. The reproducibility of sample quantity injected into the reaction channel is improved.

Other objects, advantages and salient features of the invention will become apparent to those persons skilled in the art upon reading the following detailed description, which taken in conjunction with the annexed drawings, disclosed preferred embodiments of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1a-1c are schematic representations of prior art sample injection schemes for chip based fluidic systems;

Figures 2a-2c are schematic representations of an off-column sample injector in accordance with one embodiment of the present invention;

Figures 3a-3c are schematic representations illustrating the process to form a "K-shaped" sample injector in which sample-channel and separation-channel are connected through a narrow channel in accordance with one embodiment of the present invention;

Figures 4a-4c are schematic representations of a process to form a "K-shaped" sample injector in which sample-channel and separation-channel are connected through a narrow channel and a pinhole in accordance with another embodiment of the present invention;

Figures 5a-5c are schematic representations of a process to form a "K-shaped" sample injector in which sample-channel and separation-channel are connected through a pinhole in accordance with a further embodiment of the present invention;

Figure 6 is a schematic representation of a high-throughput assay chip comprising an off-column sample injector in accordance with one embodiment of the present invention;

Figures 7a-7b are schematic representations of an off-column sample injection scheme in which the sample reservoir is not on chip, in accordance with one embodiment of the present invention;

Figure 8 is a schematic representation of an alternative off-column sample injection scheme in which the sample reservoir is not on chip, in accordance with another embodiment of the present invention;

Figure 9 is an image of a pinhole connection of two channels; and

Figures 10a-10d are images illustrating the connection between a capillary tube and a micro-channel in the chip.

DESCRIPTION OF THE ILLUSTRATED EMBODIEMNTS

The present description is of the best presently contemplated mode of carrying out the invention. This description is made for the purpose of illustrating the general principles of the invention and should not be taken in a limiting sense. The scope of the invention is best determined by reference to the appended claims.

For purpose of illustrating the principles of the present invention and not limitation, the present invention is described by reference to embodiments directed to separation in capillary electrophoresis. The present invention is equally applicable to chemical reactions, diagnostics and/or analysis in microfluidic devices. All references to separation channel hereinafter are examples of reaction channels, and are equally applicable to channels for other purposes in microfluidic devices.

Referring to one embodiment of the present invention illustrated in Figure 2a, the off-column sample injection scheme of the present invention is embodied in the form of a "K-shaped" injection device, which comprises a sample-channel **11**, a separation-channel **6**, and a narrow connection channel **12** coupling between the sample-channel **11** and the separation-channel **6**. In a particularly preferred embodiment, sample-channel **11**, separation-channel **6** and connection channel **12** are configured as in FIG. 2a. Cathode reservoir **3** and anode reservoir **4** are located at the ends of the separation-channel **6.** A sample reservoir **1** and an analyte waste reservoir **2** are located at the ends of the sample-channel **11.** The narrow connection-channel **12** connects the sample-channel **11** and the separation-channel **6** in an orthogonal configuration. The sample-channel **11** and the separation-channel **6** are substantially parallel in the region near the connection-channel **12**. At least the separation-channel **6** and/or the connection channel **12** is filled with a buffer or electrolyte, which may comprise a gel sieving matrix commonly known in the electrophoresis art.

In preferred embodiments (referring to FIG. 2b), analytes in the sample reservoir **1** are transported through the sample-channel **11** to the vicinity of the connection-channel **12**. In preferred embodiments, the analytes are transported using an electrokinetic means such as electrophoretic and/or electroosmotic forces. In other preferred embodiments, sample is transported using a pressure difference between sample reservoir **1** and analyte waste reservoir **2**.

Analytes are then introduced into the separation-channel **6** through the connection channel **12**. In preferred embodiments, the analytes are introduced using an electrokinetic means such as electrophoretic and/or electroosmotic forces. For example, a voltage is applied between sample reservoir **1** and the anode reservoir **4** or cathode reservoir **3**. As a result, analytes are then electrophoretically and/or electroosmotically injected into the separation channel at **13**. The electric field can also be applied in alternative ways such as between analyte waste reservoir **2** and the anode reservoir **4** or cathode reservoir **3**, or any other combinations between reservoirs **1**, **2**, **3** and **4** such that an electric field is created in the connection channel **12**. In other embodiments, sample may be introduced using a pressure difference between the sample-channel **11** and the separation-channel **6** without departing from the scope and spirit of the present invention (i.e., a pressure drop is created across the connection channel **12**). When the connection channel is short, diffusion force may be used for sample introduction as well. The quantity of the sample introduced depends on the analyte concentrations in the sample, the strength of the force, and the duration when this force is applied. By controlling these parameters, these methods allow introduction of a wide quantity range of analyte, from a few molecules to a few micromoles, more preferably from a few atomoles to a few nanomoles.

Referring to FIG. 2c, after analytes are introduced into the separation-channel, a voltage is applied across the buffer in the separation-channel **6** between the cathode reservoir **3** and anode reservoir **4.** The analytes introduced into the separation-channel **6** are driven down the separation-channel **6** to perform electrophoretic separation into analyte bands **14**.

The sample injected into the separation column **6** can be well controlled because the narrow channel **12** is microfabricated and can be made very short and accurately. This overcomes the problem of dimensional control for T-injectors mentioned in the Background herein. In addition, in accordance with the present invention, sample pre-separation and differential concentration may be performed in sample channel **11**. Referring to DNA sequencing, after sample channel **11** is filled with a sieving matrix, DNA fragments are electrophoresed from sample reservoir **1** through sample channel **11** to analyte waste reservoir **2**. Larger DNA fragments will be concentrated more than smaller ones at the entrance of connection channel **12**. When these analytes are injected into the separation channel **6**, differential concentration effect has been performed. When a proper loading time is selected, the fragments of interest should have reached their steady states at the entrance of connection

channel **12** while DNA template and other large molecules are still migrating in the top part of the sample channel **11**. If a sample injection is executed at this time, these large molecules will not likely enter the separation channel **6** and sample pre-separation is performed. This overcomes the augmented electrophoretic mobility-based bias problem for T-injectors mentioned in Background. Furthermore, during the process of pre-separation and differential concentration, diffusion of analytes into the connection channel **12** and separation channel **6** can be well controlled without applying any "pinched" voltages to reservoirs **3** and **4** because the connection channel **12** may be made very narrow.

In preferred embodiments, the sample-channel **11**, separation-channel **6** and connection channel **12** are micromachined onto a microchip device. The microchip can be made of glass substrates or polymeric materials. In preferred embodiments, these channels have widths less than 1 mm, more preferably between 20 μm to 200 μm. In other embodiments, the connection channel **12** is short, preferably less than 10 mm in length, more preferably between 20 μm and 500 μm, and has width less than 500 μm, and preferably between 2 μm and 50 μm. In additional preferred embodiments, the sample-channel, separation-channel and connection channel may have different widths. More preferably, the connection channel has the smallest width. In other preferred embodiments, the sample-channel has the largest width.

FIG. 3a is a schematic representation of a photomask for fabrication of a new injector of this invention. The long straight vertical line **15** is used for making the separation-channel **6**, the short horizontal line **17** for the connection channel **12**, and the curved line **16** for sample-channel **11**. In preferred embodiments, photomask line **16** is curved so that it gets close to photomask line **15** in the region where these two lines are connected by photomak line **17.** At the sections of line **16** that are far away from line **17**, the line **16** deviates from line **15**. The objective of this design is to allow sample being brought to the vicinity of the separation-channel for convenient and reproducible sample introduction, and at the same time, to ensure proper arrangement of various reservoirs in which a minimum distance is required between two adjacent reservoirs.

FIG. 3b is a schematic representation of grooves microfabricated on a substrate using the photomask presented in FIG. 3a. Sample-channel **11** and separation-channel **6** are normally connected as indicated in FIG. 3c.

In preferred embodiments (referring to FIG. 4a), a gap **18** is provided between line **15** and line **17** on the photomask. After this pattern is transferred to a substrate, various etching

methods, more preferably isotropic etching, can reduce the gap and eventually make these two channels connected, as shown in FIG. 4b and 4c. The overlap of the separation-channel **6** and the connection channel **12** can be controlled by the size of the gap and the etching parameters. If the overlap is very small, separation-channel **6** and sample-channel **11** are connected through a connection channel **12** and a pinhole **19**.

In additional embodiments (referring to FIG. 5a), only photomask lines **15** and **16** are present, and line **17** is eliminated. The gap **18** is formed by pulling line **15** closer to line **16**. After this pattern is transferred to a substrate, various etching methods, more preferably isotropic etching, can reduce the gap and eventually make these two channels connected, as shown in FIG. 5b and 5c. The overlap of the separation-channel **6** and the sample channel **11** can be controlled by the size of the gap and the etching parameters. If the overlap is very small, separation-channel **6** and sample-channel **11** are connected through only a pinhole **19**.

In other embodiments (referring to FIG. 6), a tube **20** is connected to the sample end and a tube **25** to the analyte waste end of sample-channel **11**. Tube **25** is further connected to a T-connector **26** and then to a sealed waste container **39** and then to a vacuum system **40**. An electrode **27** is introduced through one end of the T-connector to the inside of the sample-channel **11** and this end is blocked with a stopper **28** and sealed with glue **29**. The free end of tube **20** is connected either to a sample reservoir **21** or an auxiliary buffer or electrolyte reservoir **22**. (A plurality of sample reservoirs and auxiliary reservoirs may be made available for different samples and buffers.) Using the vacuum system **40**, various (or the same) sample **23** and auxiliary solutions **24** are aspirated through tube **20**, to the sample-channel **11**, and finally into the sealed waste container **39**. Tube **20** and sample-channel **11** are preferably wider than channel **12** and **6** in order to reduce the negative pressure in channel **11** and hence a flow of solution from channel **6** to channel **11**. As samples **23** pass by channel **12**, proper voltages are applied to electrode **27**, **30** and **31** so that a portion of analytes of interests in each sample is introduced into separation-channel **6** for separation. Auxiliary solution(s) **24** are used to avoid cross-contamination between samples. Putting electrode **27** at the downstream prevents bubbles generated by electrolysis from entering separation-channel **6**. The vacuum system **40** and the waste reservoir **39** may be replaced with a peristaltic pump.

The open end of the capillary tube may be moved alternatively to a sample reservoir **21** and an auxiliary solution reservoir **22**. Various sample solutions **23** are brought in series to the vicinity of the separation-channel for sample injection. High-throughput is thus achieved.

Referring to the embodiment shown in FIG. 7a, in the microchip **32**, the connection channel **12** and/or sample-channel **11** is eliminated from the microchip. Instead, a through hole **33** is provided (e.g., drilled) orthogonal to the separation-channel. There are two different modes to operate this device. Mode 1: Referring to FIG. 7b, sample is brought into the through hole **33** using tube **37**. Preferably, the through hole **33** has diameter between 20 μm and 3mm, more preferably between 200 μm and 1mm. When a solution is delivered into a hole of these dimensions, surface tension holds the solution in the hole and sample injection is carried out by applying a voltage between electrodes **30** and **31**. Then, an auxiliary electrolyte solution is brought over using tube **36** to rinse the through hole **33**. The waste solution is collected underneath the microchip and aspirated away through tube **34** by the vacuum system **40** via waste reservoir **39**. A small container **38** is sometimes used to prevent the rinsing solution from spraying out. After residual sample is rinsed out and the through hole is filled with a electrolyte solution, a voltage is applied between electrode **30** and **31** to perform separations. Mode 2: An auxiliary electrolyte solution is continuously delivered to rinse the through hole **33** and waste solution is constantly aspirated away through tube **34**. Meanwhile, a voltage is always applied between electrode **30** and **31** during operation. Samples are brought over and delivered in series into the through hole **33** using tube **37**. The sample is rinsed away shortly after it is delivered. Because the voltage is constantly applied across channel **6** and **6a**, a portion of the analytes are injected into the separation-channel **6** for separation.

In other embodiments (referring to FIG. 8), cathode reservoir **3**, electrode **30** and channel **6a** are further eliminated. Electrode **27** is incorporated through a T-connector **26**, as illustrated in FIG. 6. The two operation modes described in the previous paragraph apply to this injector as well.

In additional embodiments, multiple injectors may be arranged to inject samples to multiple separation channels on a single chip to increase the throughput (e.g., high throughput syntheses and assays).

Apparently, the sample introduction methods and apparatus disclosed in this invention can be used for other applications, in addition to electrophoretic separation. Other applications

include capillary electrochromatography, microcolumn liquid chromatography, flow injection analysis, field-flow fractionation, and integrated microfluidic systems to perform various chemical reactions and syntheses.

A variety of methods known in the art may be used to form the "K-shaped" injectors in FIG. 3, 4, and 5. For example, the chip microfabrication protocols disclosed by Liu et al. (1999), or their equivalents known in the art can be readily adapted to produce the chip component of the present invention. Alternative methods known in the art may be employed within the scope of the present invention. For example, for photolithograpy, a thin sacrificial layer of Cr/Au (300 Å Cr and 0.5 μm Au) may be deposited onto a glass wafer, followed by a photoresist coating (Shipley photoresist 1818). After soft baking at 80 °C, the photoresist may be exposed to UV radiation through a mask. The mask pattern will be transferred to the wafer after the photoresist is developed. After the exposed Cr/Au is etched off using gold and chromium etchants, the channel pattern is chemically etched into the glass. Concentrated HF may be used as the chemical etchant with an etching rate of ca. 7 μm per minute at 21 °C for borofloat glass. After etching, the residual photoresist and Cr/Au are stripped and access holes **33** if needed are drilled. The etched wafer may be thermally bonded with another wafer to enclose the grooves and form closed channels.

In one embodiment of the present invention, referring to FIG. 3a, the linewidth of line **17** is 5 μm, other lines have a linewidth of 100 μm. Line **17** connects line **15** and **16** directly. After channels are etched 20 μm in depth, the connection channel **12** has a channel-width of ca. 45 μm and all other channels have a channel-width of ca. 140 μm.

In another embodiment, referring to FIG. 4a, all lines have the same linewidth of 10 μm. There is an 60-μm-gap **18** between line **17** and **15**. All channels are etched 50 μm deep, this gap disappears, and the connection channel **12** and separation-channel **6** is connected through a pinhole **19**. FIG. 9 is an image illustrating such a pinhole connection of two channels. The pinhole may be made much smaller than that shown in the image.

In another embodiment, referring to FIG. 5a, line **16** forms a sharp angle towards line **15**. The gap between line **15** and the tip of the angle of line **16** is 60 μm. Both line have a linewidth of 10 μm. After a 50-μm-deep etching, this gap disappears, and the sample-channel **11** and separation-channel **6** is connected through a pinhole **19**.

A two-mask procedure may also be used to make the chip channels. Linewidth of both masks are 10 μm. Referring to FIG. 3a, the first photomask has only line **15** and **16** in it, and is used to make sample-channel **11** and separation-channel **6**. These channels are made 50-μm-deep. A second photomask contains only the connection channel and is used to make the connection channel **12**. The depth, normally 10 μm, of this channel can thus be controlled different from that of the sample- and separation-channel.

There are two methods to form the through hole in FIG. 7 and 8. One method is to drill hole on both wafers and then align these two holes before bonding. The preferred method is to make a chip without a through hole, then fill the channels with wax, drill the through hole, and finally warm the chip and remove the wax.

In the embodiment of FIG. 6, a capillary tube is connected to the sample channel on a microchip device. Round channels may be formed on microfabricated devices and the round channels may be used to connect capillaries to microchip devices.

When hydrofluoric acid is used for glass wafer etching, a characteristic of this etching process is that it is isotropic. Starting with a very narrow linewidth, isotropic etching results in a semicircular channel. For example, if the line width of the photomask is 5 μm, a 100-μm-deep channel is very close to a semicircle with a long radius of 102.5 μm and a short radius of 100 μm. A very "round" channel is formed after two etched wafers are face-to-face aligned and bonded. After a round channel is formed, a capillary tube may be inserted into it and secured in position with glue.

To minimize the dead volume of this connection, a two-mask process is used to fabricate semicircular channels having different radius (depth), respectively matching the inner and outer diameter of the capillary tube. The smaller channel is etched first using one mask and the larger channel second using a different mask. A round-channel having two different channel diameter is produced after aligning and bonding of the two etched wafers, as schematically disclosed in the literature. FIGS. 10a-10d are images illustrating such chip to capillary tube connection. FIG. 10a is an image of the round channels of two different diameters, FIG. 10b is an image of the round cross-section channel, FIG. 10c is image of three capillary tubes with ground tips, and FIG. 10d is the image of an assembled chip device.

The operations of the various embodiments of the present invention are controlled by a controller (not shown) to accomplish the functions recited herein. It would be within a person

skilled in the art to implement the program code given the functions and features disclosed herein.

* * *

All of the methods and apparatus disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the invention has been described with respect to the described embodiments in accordance therewith, it will be apparent to those skilled in the art that various modifications and improvements may be made without departing from the scope and spirit of the invention. For example, it will be apparent to those of skill in the art that variations may be applied to the methods and apparatus and in the steps or in the sequence of steps of the methods described herein without departing from the concept, spirit and scope of the invention. It also will be apparent that certain substance such as polymeric and ceramic materials may be substituted for the glass materials described herein to achieve the same, similar or improved results. By way of example and not limitation, the sample injection concepts of the present invention is described in connection with capillary electrophoresis in a microfabricated chip. It is understood that the present invention is also applicable to bio-separation based on other than electrophoresis, and emissive radiation based detection such as fluorescence, phosphorescence, luminescence and chemiluminescence as well as absorbance based detection. The sample introduction schemes of the present invention can be used for variety of applications, including integrated microfluidic systems for chemical analysis and sensing, and analytical separation techniques such as capillary electrophoresis, capillary electrochromatography, microcolumn liquid chromatography, flow injection analysis, and field-flow fractionation. A person skilled in the art will recognize that the instrument incorporating the essence of this invention can also be used for biomolecular analysis for DNA, proteins, carbohydrates, lipids, etc.

Furthermore, while the reaction channels in the described embodiments are defined by micro-separation channels etched in a substrate (micro-fluidics type devices or bio-chips), it is understood that the concepts of the present invention is equally applicable to columns or tubes defining the reaction channels.

All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended

claims. Accordingly, it is to be understood that the invention is not to be limited by the specific illustrated embodiments, but only by the scope of the appended claims.